

Antidiabetic effect of the aerial part ethanolic extracts of *Zygophyllum geslini* Coss. in streptozotocin induced-diabetic rats

Houria Medjdoub, Boufeldja Tabti

Laboratoire de recherche sur les Substances Naturelles et Bioactives, Université de Tlemcen, Algérie

(Received 22 September 2011, revised 18 May 2012, accepted 15 October 2012)

ABSTRACT

Zygophyllum geslini Coss commonly called Aggaya is a zygophyllaceae very widespread in the Algerian Sahara. The present study investigate the antidiabetic effect of the ethanolic extract of *Z. geslini* aerial part administered orally in Wistar rats rendered diabetic by the intravenous injection of 50 mg/kg of streptozotocin. The present study consists of two essential parts. The first realized over a long period was devoted to the study of the ethanolic extract effect on glycemia and body weight during five weeks. In this first series of experiments, a non significant reduction of glycemia was noticed. The second part consists of the follow-up of the variation of the glycemia after treatment by the same extract during 7 hours. A significant reduction in glycemia was observed between the second and seventh hour after administration of 1.0 g/kg ethanolic extract. This reduction can go up to 75 % of basal glycemia. Thus, the ethanolic extract at 1 g/kg is endowed with a remarkable antidiabetic activity. This extract may thus represent a natural resource of new antidiabetic substances.

KEY WORDS: *Zygophyllum geslini* Coss.; Streptozotocin; Ethanolic extract

INTRODUCTION

Diabetes mellitus is a group of disorders with different aetiologies. It is characterized by derangements in carbohydrate, protein and fat metabolism caused by the complete or relative insufficiency of insulin secretion and/or insulin action (1). Chronic hyperglycaemia causes secondary complications affecting eyes, kidney, nerves and arteries (2). For example, the morbidity and mortality associated with retinopathy, nephropathy, neuropathy and cardiovascular diseases remain the leading cause of death in type 2 diabetes mellitus. Indeed, control of hyperglycaemia is essential. This involves exercise, diet and current therapeutic agents including sulfonylureas and related compounds, biguanides, thiazolidenediones, α -glucosidase inhibitors and insulin (3). However, the therapeutic agents are either too expensive or have undesirable side effects and contraindications (4). From now on, plants used in traditional medicines to treat diabetes mellitus represent a valuable alternative for the control of this disease in many countries (5, 6). Because of their perceived effectiveness, minimal side effects in clinical experience and relatively low cost, herbal drugs are prescribed widely even when their biologically active compounds are unknown (7). This leads to increasing search for improved antidiabetic drugs, when the World Health Organisation has recommended that this area warrants attention and a novel strategy is in the aim to maximise the possibility of plants medicine (8). Since ancient time, plants extracts have been used in traditional medicine for the treatment of diabetes mellitus.

In Algeria, several plants are known to have antidiabetic proprieties notably the genus *Zygophyllum*. One is *Zygophyllum geslini* Coss (9) which is the aim of this work. *Zygophyllum geslini* (Zygophyllaceae) commonly called Aggaya, is very widespread in the Algerian Sahara (10). This species is known locally to treat diabetes mellitus (9). In the present work, the glucose-lowering effect of the ethanolic extract of the *Z. geslini* aerial part in

normal and streptozotocin-induced diabetic rats was evaluated.

MATERIALS AND METHODS

Plant material and extraction

Fresh aerial parts of *Z. geslini* were collected in August from Adrar (South of Algeria). Authentication of the plant was carried out by Dr. A.MAHBOUBI and A. BELASKRI, Department of Biology, Faculty of Sciences, University of Tlemcen, Algeria and the voucher specimen of the plant has been retained in the Department of Biology.

The plant material was dried in the laboratory at room temperature and powdered in a mixer grinder. Twenty grams of powdered dried were macerated in 100 ml of ethanol for 24 hours at room temperature. After filtration the filtrate was evaporated under vacuum. The resulting extract (2-3g) was dissolved in Tween 80 at 5% (w/v)..

Chemical analysis of the ethanolic extract

The ethanolic extract was tested for the presence of different families of compounds according to methods previously described (11, 12).

Toxicity evaluation

The ethanolic extract was tested for its acute toxicity in rats. Different doses of the drug (0.45, 0.9, 1, 11, 12, and 15 g/kg) were administrated orally to different groups of rats (4 rats/group). Control groups received Tween 80 at 5 %.

Animals

Male Wistar rats weighing 215-275 g from the animal house of the faculty of Science, University of Tlemcen, were used. The animals were fed with standard laboratory diet and given water *ad libitum*. Prior to the experiment, the animals were subjected to fast for 16 hours with free access to water.

Streptozotocin-induced diabetic rats

Diabetes was induced in fasted rats by tail vein injection of streptozotocin (STZ: 50 mg/kg, *iv*) dissolved in 0,1 M citrate buffer (pH 4,5). Fasted blood glucose level were assessed 48 hours after STZ injection as well as glucosuria to confirm the diabetic state. Only rats with a fasting blood glucose level at least 2.0 g/l and positive urine glucose were used in the experiments (13). Such experiments were conducted three days after streptozotocin administration.

Collection of blood and determination of serum glucose level

Blood samples were drawn by retro-orbital puncture. The serum was separated by blood centrifugation at 3000g for 15min and the glucose level was measured by the glucose-oxidase method previously described (14).

Experimental set up

Three sets of experiments were carried out; short-term and long-term study and oral glucose tolerance test. In both the short-term and long-term study, animals were divided into four groups of five rats each as follows : normal control rats, normal treated rats, diabetic control rats and diabetic treated rats. The control animals received the same volume of Tween 80 as the treated rats.

Short term study

In the short-term study, the treated rats received 1.0 g/kg of the ethalonic extract, blood samples being collected before and up to the 7th hour after administration of the ethanolic extract.

Long term study

The treated rats received 0.5 g/kg of the ethanolic extract once a day during five weeks. Fasting serum glucose levels, as well as body weight, were measured at weekly intervals..

Oral glucose tolerance test

Overnight fasted normal rats were divided into 2 groups of five rats each. One group kept as control received 5% of Tween 80 (*p.o*). The second group received the ethanolic extract (1.0 g/kg, *p.o*). One hour later, the rats were loaded with glucose (3 g/kg). Serum glucose levels were measured at 0, 30, 60, 90 and 120 min after glucose loading.

Statistical analysis

Results were expressed as mean values ± SEM. Differences between groups were considered to be significant at *P* < 0,05 using unpaired Student's 't' test.

RESULTS

Chemical analysis of the ethanolic extract

Qualitative tests for the presence of various classes of compounds in the ethanolic extract documented the presence of tannins, saponins, flavonoids, coumarines, volatile oils and fatty acids.

Toxicity evaluation

The ethanolic extract of *Z. geslini* did not show any mortality at dose up to 15 g/kg indicating that it is not toxic by oral administration in male Wistar rats.

Short-term study

Table 1 refers to the short-term experiments. In the normal rats, there was little to distinguish between control and treated animals, the serum glucose concentration being only somewhat lower (*p*<0.05) in the latter animals than in the former ones 3 and 5 hours after administration of the ethanolic extract.. The serum glucose concentration remained fairly stable in the diabetic control rats. However, in the diabetic treated rats, a progressive decrease in serum glucose concentration was observed.. Such a decrease amounted to about 80 % at the seventh hour. .

Table 1: Short-term effect of the ethanolic extract on serum glucose concentration (g/l) over 7 hours.

Time (hours)	0	1	2	3	5	7
Normal control	1.03±0.07	0.95±0.02	0.91±0.06	0.97±0.04	0.86±0.04	0.74±0.02
Normal treated	0.95±0.08	0.99±0.02	0.81±0.01	0.85±0.02*	0.68±0.05*	0.87±0.14
Diabetic control	3.78±0.33	3.75±0.45	3.62±0.38	3.41±0.36	4.05±0.28	3.08±0.05
Diabetic treated	2.94±0.54	2.63±0.34	2.33±0.39*	2.06±0.08*	0.83±0.06**	0.61±0.06****

*: *P*<0,5
 **: *P*<0,01
 ****: *P*<0,001

Long term study

The effect of the ethanolic extract on the fasting serum glucose level of normal and diabetic rats is documented in Table 2. No obvious change was noticed in the normal rats, whether in control or treated animals. In the diabetic rats, however, the serum glucose concentration was lower in the treated animals than in the control animals.

The body weight of the normal rats progressively increased over the 5- week period. The paired difference between the first and last measurement averaged, in the control and treated normal rats respectively, 21.6 (n=5)

and 16.5 (n=5), yielding an overall mean value of 19.0... (n=10). The initial body weight was already lower (p<0.02) in the diabetic rats (223.5 ± 7.8 g; n=10) than in the normal rats (249.1 ± 5.6 g; n=10). Moreover, the diabetic rats failed to gain weight over the subsequent 5-week period.

Oral glucose tolerance test

The effect of the ethanolic extract on serum glucose level during an oral glucose tolerance test conducted in normal fasted rats is outlined in table 4. The prior administration of the ethanolic extract failed to improve glucose tolerance .

Table 2: Long-term effect of the ethanolic extract on the glycaemia (g/l) during the five weeks.

Time (week)	0	1	2	3	4	5
Normal control	0.91± 0.03	0.96± 0.02	1.04± 0.07	0.95± 0.07	1.23± 0.1	0.84± 0.05
Normal treated	0.94 ± 0.06	1.10 ± 0.8	1.08 ± 0.07	0.91 ± 0.02	1.13 ± 0.1	0.88 ± 0.12
Diabetic control	4.18± 0.56	2.24± 0.84	3.24± 0.98	3.01± 1.13	3.94± 1.19	5.11± 1.09
Diabetic treated	3.68± 0.16	1.98± 0.80	2.94± 0.71	2.00± 0.36	2.99± 0.79	2.67± 1.45

Table 3: Effect of the ethanolic extract on the body weight (g) during the five weeks.

Time (week)	0	1	2	3	4	5
Normal control	250± 9	255±10	259±9	263±12	269±12	272±12
Normal treated	247±7	247±7	250±9	254±10	261±10	264±10
Diabetic control	223±9	215±11	221±14	222±15	216±13	218±12
Diabetic treated	223±13	225±12	228±15	227±18	214±21	202±22

Table 4: Effect of the ethanolic extract on the oral glucose tolerance (glycaemia g/l).

Times (min)	0	30	60	90	120	
Normal control		1.12±0.40	1.35±0.08	1.33±0.09	1.23±0.05	1.19±0.02
Normal treated 1 hour before the test		0.86±0.05	1.68±0.22	1.71±0.22	1.59±0.23	1.63±0.16

DISCUSSION

Zygophyllum. geslini is a plant widely used in Algerian folk medicine for the treatment of diabetes mellitus. The antidiabetic effect of the ethanolic extract from the aerial part of this plant was investigated in the present study using the STZ experimental model of diabetes mellitus. STZ-induced hyperglycemia has been described as a useful experimental model to study the activity of antidiabetic agents (15-17).

The results obtained in this research show that the ethanolic extract is endowed with antihyperglycaemic activity. A good response of the animals to the treatment was observed.. At 1 g/kg of the extract the response of the rats was highly significant, a reduction of about 80% of the

basal serum glucose concentration was observed 7h after administration of the extract. This represents a interesting result. In normal rats, however, the administration of the extract failed to improve glucose tolerance.

In long-term experiments, the dose of 500 mg/kg did not give significant results. This could be explained by the fact that the diabetes induced by STZ (50 mg/kg) passes by different stages during the weeks which follow the injection of the diabetogenic agent (17).

Zygophyllum gaetulum was previously investigated for the antidiabetic activity of its aqueous extract in alloxan-induced diabetic rats. The results have shown a significant reduction of blood glucose level in rats and in patients with diabetes mellitus (18, 19).

Our study remains preliminary and not very indicative on the exact mechanism of the ethanolic extract action. It could be postulated that the plant extract contains compounds which would act in competition with insulin (see glucose tolerance test). This could be compared with the properties of cystein derivatives which constitute the active compounds of *Allium sativum* and *A. cepa* (4, 6, 20). Moreover, these compounds decrease the glycemia in fasted animals but not in the post-prandial period.

In conclusion, *Z. geslini* has an interesting antihyperglycemic effect, which merits further investigations. This effect could be attributed to the chemical compounds of the plant, e.g. the saponins or the phenolic compounds. The present research remains a contribution and requires a continuation with *in vitro* and *in vivo* experiments to isolate the active ingredient and for a good understanding of its mode of action.

REFERENCES

1. Guerci B, Bohme P, Kearney-Schwartz A, Zannad F, Drouin P: Endothelial dysfunction and type 2 diabetes. *Diabetes Metab* 27: 436-447, 2001
2. Raccach D: Epidémiologie et physiopathologie des complications dégénératives du diabète sucré. *EMC-Endocrinologie* 1: 29-42, 2004
3. Mahler RJ, Adler ML: Type 2 Diabetes Mellitus: Update on Diagnosis, Pathophysiology, and Treatment. *Journal of Clinical Endocrinology and Metabolism* 84:1165-1171, 1998
4. Dey Lucey MD, Anoja S, Attele DDS, Chun-Su Yuan MD: Alternative therapies for type 2 diabetes. *Alternative medicine Review* 7: 45-58, 2002
5. Jouad H, Haloui M, Rhiouani H, El Hilaly J, Eddouks M: Ethnobotanical survey of medicinal plants used for the treatment of diabetes, cardiac and renal diseases in the north centre region of Morocco (Fez-Boulemane). *J of Ethnopharmacology* 77: 175-182, 2001
6. Al-Achi A: Herbs that affect blood glucose levels. *Women's Health in Primary Care* 8: 325-330, 2005
7. Niyah Njike G, Watcho P, Nguenefack TB, Kamanyi A: Hypoglycaemic activity of the leaves of *Bersama engleriana* in rats. *Afr J Trad* 2: 215-221, 2005
8. WHO: Stratégie de l'OMS pour la médecine traditionnelle pour 2002-2005. WHO /EDM /TRM/2002.1
9. Smati D, Longeon A, Guyot M: 3 β -(3, 4-Dihydroxycinnamoyl)-erythrodiol, a cytotoxic constituent of *Zygophyllum geslini* collected in the Algerian Sahara. *J of Ethnopharmacology* 95: 405-407, 2004
10. Ozenda P: *Flore du Sahara*. (Ed) du Centre National de la Recherche scientifique, Paris, pp 318-320, 1977
11. Karumi Y, Onyeyili PA, Ougubuaja VO: Identification of active principles of *M. balsamina* (Balsam Apple) leaf extract. *J Med Sci* 4: 179-182, 2004
12. Edeoga HO, Okwu DE, Mbaebie BO: Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology* 4: 685- 688, 2005
13. Crouch R, Kimsey G, Priest DG, Sarda A, Buse MG: Effect of streptozotocin on erythrocyte and retinal superoxide dismutase. *Diabetologia* 15: 53-57, 1978
14. Trinder P: Determination of glucose in blood using glucose oxidase with an alternative oxygen receptor. *Ann Clin Biochem* 6: 24-27, 1969
15. Szkudelski T: The mechanism of alloxan and streptozotocin action in B cells of the rats pancreas. *Physiol Res* 50: 536-546, 2001
16. Chen V, Ianuzza CD: Dosage effect of streptozotocin on rat tissue enzyme activities and glycogen concentration. *Can J Physio. Pharmacol* 60: 1251-1256, 1981
17. Junod A, Lambert AE, Stauffacher W, Renold AE: Diabetogenic action of streptozotocin: relationship of dose to metabolic response. *J of Clinical Investigation* 48: 2129-2139, 1969
18. Jaouhari JT, Lazrek HB, Jana M: Hypoglycaemic response to *Zygophyllum gaetulum* extracts in patients with Non-Insulino-Dependent Diabetes Mellitus. *J of Ethnopharmacology* 69: 17-20, 2000
19. Jaouhari JT, Lazrek HB, Seddik A, Jana M: The hypoglycaemic activity of *Zygophyllum gaetulum* extracts in alloxan-induced hyperglycaemic rat. *J of Ethnopharmacology* 64: 211-217, 1999
20. Marles RJ, Farnsworth NR: Plants as sources of antidiabetic agents. *Econ Med Plant Res* 6: 149-187, 1994

Corresponding author: Medjdoub H, Laboratoire de recherche sur les Substances Naturelles et Bioactives, Université de Tlemcen, Algérie. E-mail : doc_algerie@yahoo.fr